

GD2-mediated impairment of macrophage phagocytosis drives pulmonary metastasis in osteosarcoma

Yunfei He^{1,2,#}, Peng Yang^{1,#}, Peng Ding^{3,#}, Zeng Zhang^{3,#}, Jichuan Wu⁴, Chunjie Wang⁵, Guanghui Hou⁵, Jun Ge¹, Quan Zhou¹, Zhuorun Song¹, Huilin Yang^{1,*}, Tao Liu^{1,*}, Shunyi Lu^{1,*}

Affiliations

¹ Department of Orthopedics, The First Affiliated Hospital of Soochow University, Suzhou 215006, China

² Institutes for Translational Medicine, The First Affiliated Hospital of Soochow University, Suzhou, 215123, China

³ Department of Orthopedics, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, 200233, China.

⁴ Biomedical Polymers laboratory, college of chemistry chemical engineering and Materials Science and State Key laboratory of Radiation Medicine and Protection, Soochow University, Suzhou 215123, China

⁵ Institute of Functional Nano & Soft Materials (FUNSOM), Jiangsu Key Laboratory for Carbon-Based Functional Materials & Devices, Soochow University, Suzhou, 215123, China

* Corresponding author. Email: hlyang@suda.edu.cn; liutao8250@suda.edu.cn; lushunyi@suda.edu.cn.

These authors contributed equally to this work.

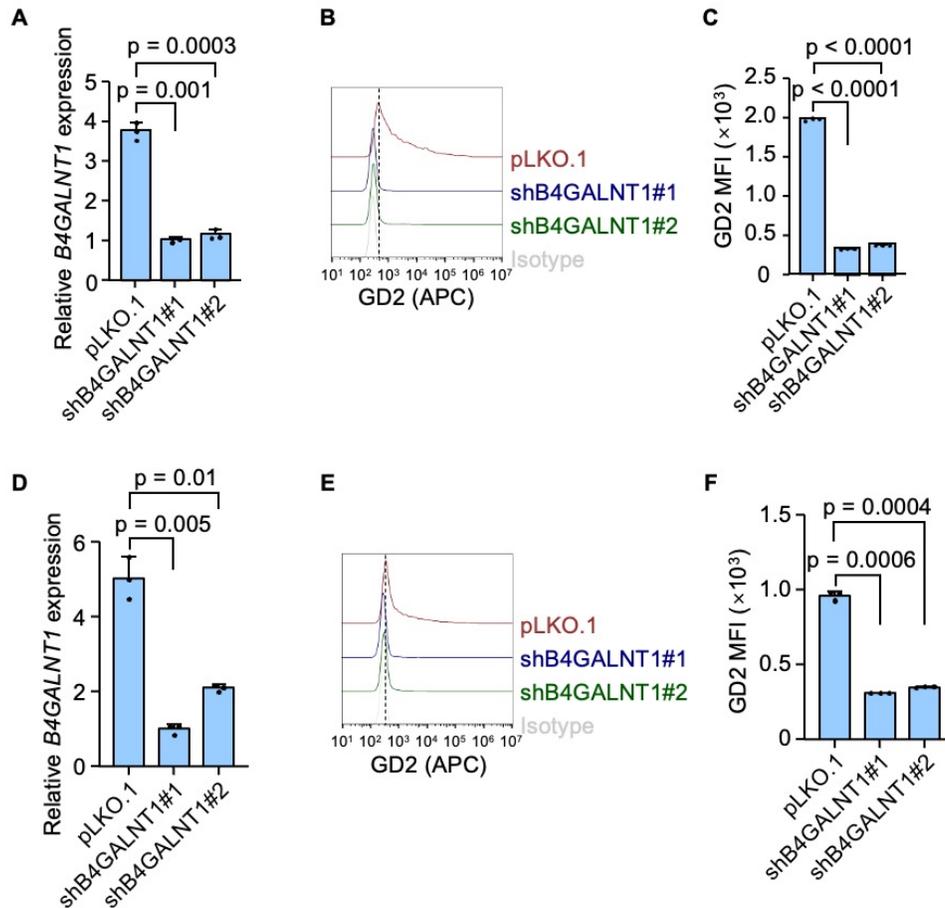


Figure S1. GD2 depletion in different osteosarcoma cell lines. **(A-C)** GD2 depletion in U2OS. Shown are quantitative PCR analysis to validate *B4GALNT1* knockdown **(A)**, Flow cytometric analysis of GD2 expression in U2OS with or without *B4GALNT1* knockdown **(B)** and quantitation of GD2 mean fluorescence intensity (MFI) **(C)**. **(D-E)** GD2 depletion in 143B. Shown are quantitative PCR analysis to validate *B4GALNT1* knockdown **(D)**, Flow cytometric analysis of GD2 expression in U2OS with or without *B4GALNT1* knockdown **(E)** and quantitation of GD2 mean fluorescence intensity (MFI) **(F)**. P values were obtained by 2-tailed unpaired t test **(A, C, D and F)**. Data are represented as mean ± SD.

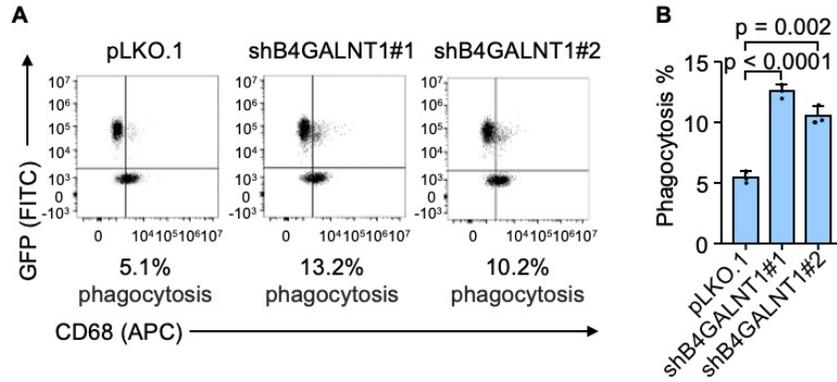


Figure S2. GD2 suppresses phagocytosis of osteosarcoma cells. **(A, B)** Representative flow cytometry plots depicting the phagocytosis of GFP-labeled U2OS cells (with or without *B4GALNT1* knockdown) co-cultured with THP-1 derived macrophages **(A)** and flow-cytometry-based quantification of phagocytosis of U2OS in the presence of THP-1 derived macrophages **(B)**. P values were obtained by 2-tailed unpaired t test **(B)**. Data are represented as mean \pm SD.

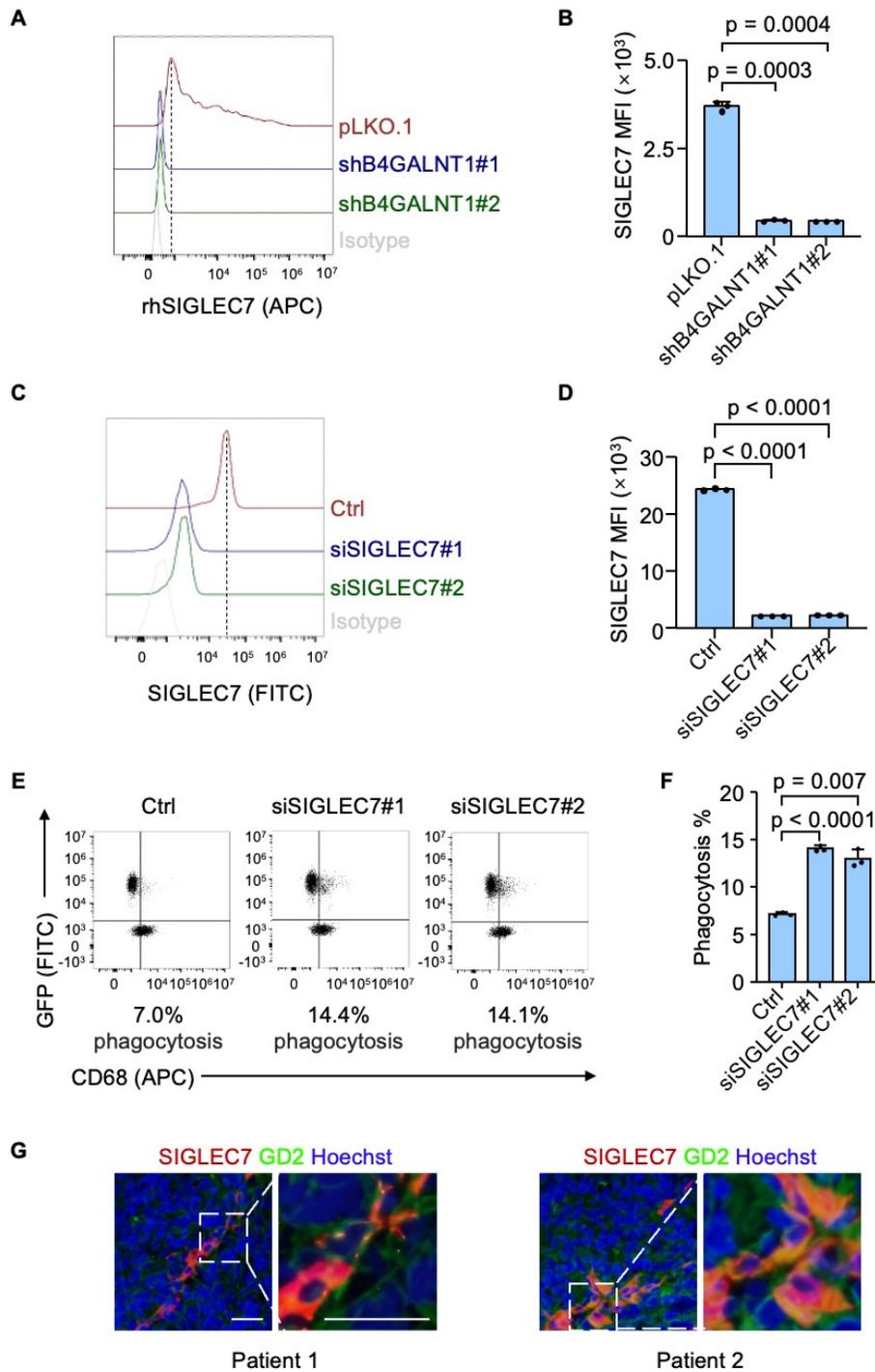


Figure S3. GD2 functions by interacting with SIGLECs. **(A)** Flow cytometric histograms of U2OS (with or without *B4GALNT1* knockdown) stained with recombinant human SIGLEC7. **(B)** Quantitation of SIGLEC7 mean fluorescence intensity (MFI) in panel **(A)**. **(C)** Flow cytometric analysis of SIGLEC7 expression in THP-1 (with or without *SIGLEC7*

knockdown). **(D)** Quantitation of SIGLEC7 mean fluorescence intensity (MFI) in panel **(C)**. **(E, F)** Representative flow cytometry plots depicting the phagocytosis of GFP-labeled U2OS cells co-cultured with THP-1 derived macrophages (with or without *SIGLEC7* knockdown) **(E)** and flow-cytometry-based quantification of phagocytosis of U2OS in the presence of mouse bone marrow derived macrophages**(F)**. **(G)** SIGLEC7 and GD2 immunostaining of lung metastases from osteosarcoma patients. Scale bars: 25 μm . P values were obtained by 2-tailed unpaired t test **(B, D and F)**. Data are represented as mean \pm SD.

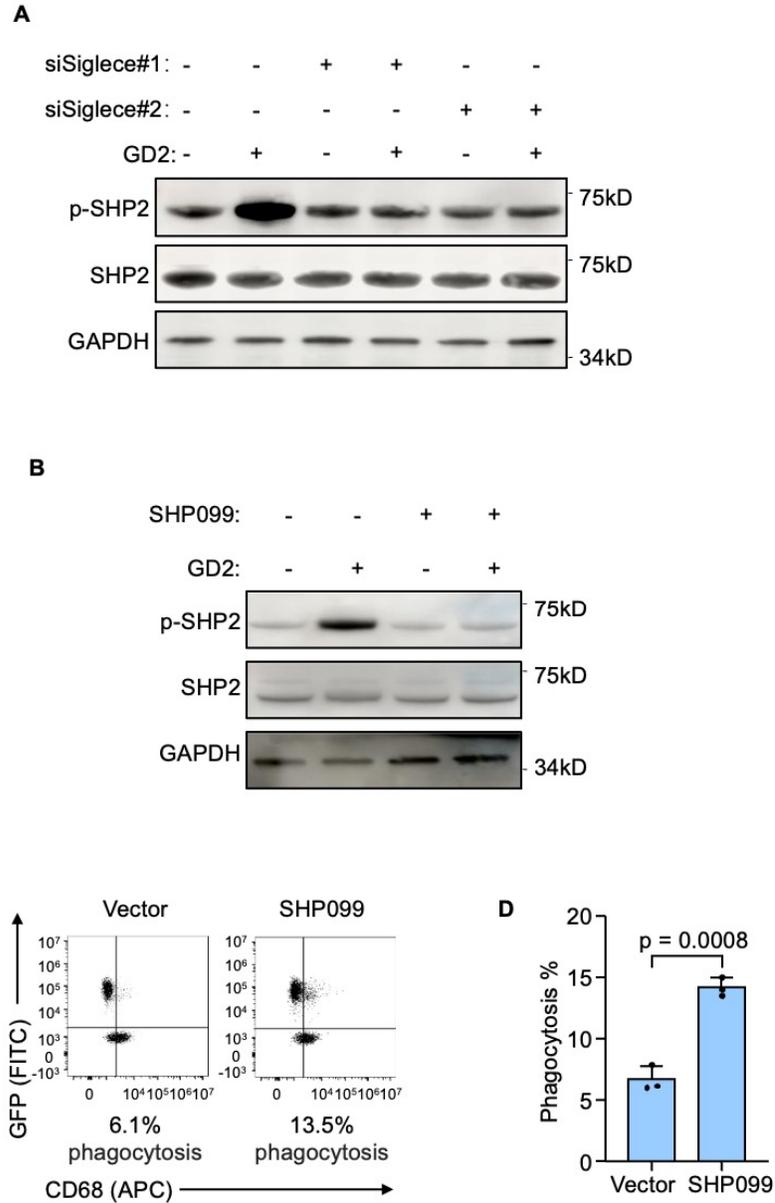


Figure S4. GD2 activates SH2-containing protein tyrosine phosphatase 2. **(A)** Western blot analysis of phosphorylated SHP2 protein level in mouse bone marrow derived macrophages (with or without *Siglece* knockdown) after treated with GD2. **(B)** Western blot analysis of phosphorylated SHP2 protein level in THP-1 derived macrophages after treated with GD2 and/or SHP099. **(C, D)** Representative flow cytometry plots depicting the phagocytosis of GFP-labeled U2OS cells co-cultured with THP-1 derived macrophages (with or without SHP099 treatment) **(B)** and flow-cytometry-based quantification of phagocytosis of U2OS in the presence of mouse bone marrow derived macrophages **(C)**.

P values were obtained by 2-tailed unpaired t test (**D**). Data are represented as mean \pm SD.

Fig. 5A

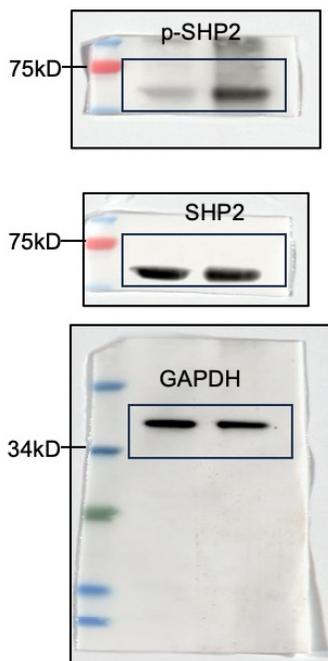


Fig. 5D

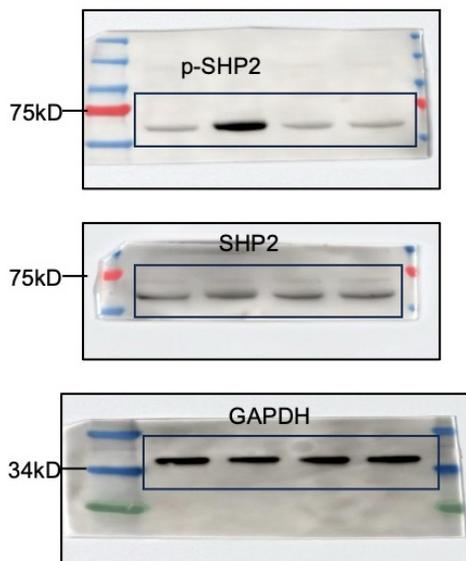


Fig. S3B

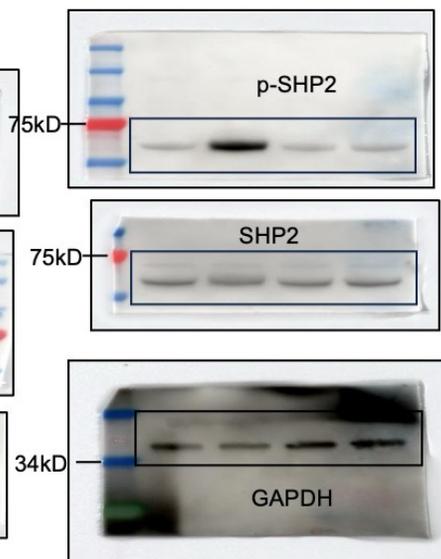


Fig. S3A

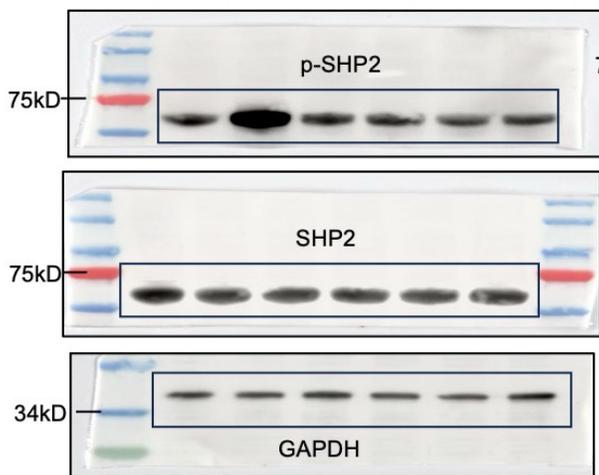


Figure S5. Uncropped gels.